

Applicant: THUNNISSEN *et al.*
Serial No.: 10/069,689
Filing Date: February 21, 2002
Second Supplemental Preliminary Amendment
July 9, 2004
Page 2 of 5

Amendments to the Claims:

This listing of claims will replace all prior versions, and listings, of claims in the application:

Listing of Claims:

1. (currently amended) A method of detecting the presence of a nucleotide sequence within a double-stranded DNA in a sample, said method comprising the following steps:
 - a. coating a solid glass support with a first layer of biotinylated serum albumin in an amount to create sufficient binding sites for capture probes, drying said first layer to ~~form a~~ and incubating said first dried layer ~~with streptavidin to form and incubating said first dried layer with~~ a second homogenous layer of streptavidin having sufficient density to perform efficient microarray analysis;
 - b. digesting a double-stranded DNA with an exonuclease to convert said double-stranded DNA to single-stranded DNA;
 - c. capturing a first nucleic acid probe adapted by biotin to said coated solid glass support;
 - d. hybridizing (i) the single-stranded DNA with the first nucleic acid probe, and (ii) one or more second nucleic acid probes, wherein said second nucleic acid probe is labeled with a detectable moiety and can hybridize with the single-stranded DNA adjacent the hybridized first nucleic probe;
 - e. ligating the hybridized first and second nucleic acid probes in case of a perfect match;
 - f. denaturing the ligated first and second nucleic acid probes from the single-stranded DNA to which they were hybridized;
 - g. removing labeled probes that are not covalently bound and single stranded DNA; and

Applicant: THUNNISSEN *et al.*
Serial No.: 10/069,689
Filing Date: February 21, 2002
Second Supplemental Preliminary Amendment
July 9, 2004
Page 3 of 5

h. detecting a detectable moiety that has been ligated to said first nucleic acid probe indicating the presence of the nucleotide sequence within the double-stranded DNA in the sample;

wherein steps c.-h. are performed by microarray technique.

2. (previously presented) The method of claim 1, wherein said one or more second nucleic acid probes comprise a mixture of partly randomized probes to allow detection of mutations without knowing the site and type of mutation beforehand.

3. (previously presented) The method according to claim 1, wherein said solid glass support is made of Starfrost glass.

4. (previously presented) The method of claim 1, wherein the one or more first nucleic acid probes are placed on said solid glass support by light-directed oligonucleotide synthesis.

5. (previously presented) The method of claim 1, wherein the detectable moiety on the second nucleic acid probe is digoxigenin, and the detecting step is performed by binding the digoxigenin with anti-digoxigenin antibody fragments.

6. (previously presented) A device suitable for carrying out the detection method of claim 1, which comprises a solid glass support having a first layer of biotinylated serum albumin and a second layer comprising streptavidin, said layers having sufficient density to perform efficient microarray analysis.

7. (previously presented) The device according to claim 8, wherein said solid glass support is made of Starfrost glass.

Applicant: THUNNISSEN *et al.*
Serial No.: 10/069,689
Filing Date: February 21, 2002
Second Supplemental Preliminary Amendment
July 9, 2004
Page 4 of 5

8. (previously presented) A kit comprising:

- a. a device suitable for carrying out the detection method of claim 1;
- b. a first nucleic acid probe which binds to target DNA and which is adapted with a capture moiety; and
- c. a second nucleic acid probe which binds to target DNA adjacent the first probe and which is labeled with a detectable moiety.

9. (previously presented) A method for organizing microarray analysis on a solid support for rapid visual detection of abnormalities which comprises arranging a duplicate set of probes where a first series of arrays are for wild-type mutation order and a second series of arrays are for the classical sequencing order, wherein the microarray analysis is conducted using the method of claim 1.

10. (previously presented) The method of claim 1, wherein prior to drying said first layer in step a, Parafilm covered by a weight, or a surfactant is employed to enhance the distribution of said first layer.

11. (previously presented) The device of claim 6, wherein the solid glass support is obtained by prior to drying said first layer in step a, Parafilm covered by a weight or a surfactant is employed to enhance the distribution of said first layer.

12. (previously presented) The kit of claim 8, further comprising an exonuclease.

13. (previously presented) The kit of claim 8, further comprising a ligase.

14. (previously presented) The kit of claim 8, further comprising an exonuclease and a ligase.